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# EXPEDITED PROCEDURE - RESPONSE AFTER FINAL

DATE: November 10, 2003

FROM: Kathleen D. Rigaut, Ph.D., J.D.

DELIVER TO: Examiner Li
Art Unit 1632
Fax number (703) 872-9306

RE: U.S. Patent Application No. 09/487,851

Total Pages (including this cover) 43

***Examiner Li:***

***As per our telephone conference I am faxing a copy of a Rule 131 Declaration in connection with the outstanding official action in the above-identified patent application.***
***Thank you for your attention to this matter.***

***Respectfully submitted,***
***Kathleen D. Rigaut, Ph.D., J.D.***
***Reg. No. 43,047***

***IF THE ACCOMPANYING/ABOVE MESSAGE IS NOT RECEIVED PROPERLY, PLEASE CALL (215) 563-4100 AND LEAVE A MESSAGE FOR THE OPERATOR INDICATED ABOVE.***

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

| | | |
|---|---|---|
| In re the Application of | ) | Group Art Unit: 1632 |
| Robert J. Levy et al. | ) | Examiner: Li, Qian J |
| Serial No. 09/487,851 | ) | Response to Paper No.29 |
| Filed: January 19, 2000 | ) | |
| For: "Reverse Gene Therapy" | ) | |

**DECLARATION OF ROBERT J. LEVY**

I, Robert J. Levy, hereby declare that:

1. I am a citizen of the United States and reside at 440 Merion Road, Merion Station, PA 19066.

2. I received a Bachelor's degree from Washington University and an M.D. from Johns Hopkins School of Medicine. The details of my education and professional history are set forth in my curriculum vitae, attached hereto as Exhibit A.

3. I have over 33 years experience in the field of medicine, my particular area of expertise being in cardiovascular disease.

4. I am the author or co-author of more than 145 scientific articles on the subjects of gene therapy, cardiac therapy, and heart valve disease. A list of these articles is set forth in my curriculum vitae, attached hereto. My current area of research involves utilizing reverse gene therapy to treat cardiac arrhythmias. I have discovered that administration of a hMiRP1 ion channel mutant has a positive

therapeutic electrophysiologic effect that could be used in treating re-entrant atrial flutter.

5. I am an inventor of the subject matter disclosed and claimed in U.S. Patent Application Serial No. 09/487,851, entitled "Reverse Gene Therapy." (hereinafter "the '851 application").

**Statements Regarding Adequacy of the Disclosure to Enable Practice of the Invention**

6. I have read and am familiar with the Official Action dated February 27, 2003, in the '851 application. I understand the nature of the rejection made by the Examiner concerning adequacy of the disclosure to enable one skilled in the art to practice the invention.

7. As exemplified in the specification, I have discovered that administration of a mutant HERG gene to the cardiac tissue produces delayed cardiac repolarization. Various means of practicing this method, as well as evidence of it's efficacy are disclosed throughout the specification. For example, pages 11-13 of the specification discuss the above gene, and mutants thereof (Q9E-hMiRP1), which are effective in preventing re-entrant atrial flutter. Similarly, page 13 of the specification describes the Q9E-hMIRP mutation in the MIRP gene, which interferes with the physiological function of HERG. When a MIRP mutant is provided to the atrial myocardium of a subject with re-entrant atrial flutter, the conductivity of the atrial tissue would be thereby decreased, and the disorder is alleviated.

Various means of administering these mutant HERG genes are discussed throughout the specification, and are specifically described at pages 19-23 of the specification. Additionally, Example III of the specification provides detailed methods of administering a mutant hMiRP1 gene to achieve the equivalent of Class III anti-arrhythmic activity.

8. Further evidence of the efficacy of administration of mutant hMiRP1 to treat re-entrant atrial flutter is provided herewith as Exhibit B. This evidence demonstrates that a mutant (Q9E-hMiRP1) transgene can be used to mimic class III anti-arrhythmic effects, and that these effects can be limited to a specific area of the atrial myocardium to disrupt regional re-entrant arrhythmia pathways.

Individuals who carry the Q9E-hMiRP1 variant exhibit diminished potassium currents, resulting in delayed myocardial repolarization following clarithromycin administration.

Exhibit B demonstrates that administration of a gene therapy vector comprising the Q9E-hMiRP1 variant to the atrial myocardium, followed by clarithromycin injection induces waveform changes and prolongation of the atrial epicardial monophasic action potential (MAP) duration. The MAP duration increases with length of clarithromycin administration in Q9E mutant but not wild type pigs.

The hMiRP1 and Q9E-hMiRP1 plasmids were created by subcloning the full-length coding sequence of the hMiRP1 potassium channel and the missense mutation, Q9E-hMiRP1 into the BAMHI/SACI sites of the pIRES2-eGFP bicistronic expression vector from Stratagene (LaJolla, CA). DAC heteroplexes were generated using an optimized formulation consisting of 10 mg of GFP plasmid DNA ("D") mixed with 10mg of mouse monoclonal anti-

bovine DNA IgM (U.S. Biological, Swampscott, MA) ("A") in a total volume of 50 μl PBS, followed by incubation at 37°C for 1 hour. 5 ml of cationic lipid ("C"), composed of a 1:1 (w/w) formulation of N-[1-(2,3-dioleyloxy)propyl]-n,n,n-triethylammonium chloride (DOTMA, Sigma Chemical Co., St.Louis, MO) and dioleoyl phosphatidylethanolamine (DOPE, Sigma) was added to DA with vortexing to form DAC. The heteroplex (DAC) was incubated at room temperature for 35 minutes or more before use.

However, initial DNA injection studies used only plasmid DNA ("naked DNA", uncomplexed) in pig atrial myocardial injection studies using hMiRP1, or Q9E-hMiRP1 plasmids. Following a right thoracotomy under general anesthesia, a series of pigs were subjected to atrial myocardial injections with DAC preparations using either hMiRP1, or Q9E-hMiRP1 plasmids. Then the waveform changes were measured at various time points after clarithromycin infusion. (See attached protocol and figures) Q9E-MiRP1 transfection plus clarithromycin was the model therapeutic approach investigated in these studies, because of the comparable mechanisms of action to Class III anti-arrhythmics, that also result in diminished potassium channel currents. Therefore, the $I_{Kr}$ response of transgene Q9E-hMiRP1 to clarithromycin demonstrated in the present studies may be used to control regional atrial re-entrant arrhythmia activity. This strategy is also attractive since the electrophysiologic effects of over expressed Q9E-hMiRP1 can be modulated with variable dosing of clarithromycin or its analogues. Additionally, other potassium channel mutations such as the dominant negative HERG mutation, A561V, should also yield promising results as candidate gene therapy constructs. A brief summary of these

methods is provided in Exhibit B.

9. Taken together, the teachings in the specification, and these experimental results clearly demonstrate the adequacy of the disclosure of the '851 application to enable anyone skilled in the art to practice the methods of the claimed invention without undue experimentation.

10. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so make are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful statements may jeopardize the validity of the above-referenced application or any patent issued thereon.

11/8/03
DATE

Robert J. Levy

R. Levy
Septebmer 2003
CONFIDENTIAL

# CURRICULUM VITAE


## Robert J. Levy, M.D.

Home Address: 440 Merion Road
Merion, PA 19066

Office Address: The Children's Hospital of Philadelphia
Abramson Pediatric Research Center, Room 1107B
34th & Civic Center Boulevard
Philadelphia, PA 19104-4399

Social Security Number: 498-46-1620

Education:

| | | |
|---|---|---|
| 1966 | B.A. | Washington University, St. Louis |
| 1970 | M.D. | Johns Hopkins University School of Medicine, Baltimore |
| 1998 | M.A. | University of Pennsylvania (Honorary) |

Postgraduate Training and Fellowship Appointments:

| | |
|---|---|
| 1970-71 | Intern, Children's Hospital of Pittsburgh, Pittsburgh |
| 1971-73 | Resident, The Johns Hopkins Hospital, Department of Pediatrics, Baltimore |
| 1975-78 | Fellow in Cardiology, Children's Hospital Medical Center, Boston |
| 1975-78 | Clinical Fellow in Pediatrics, Harvard Medical School, Boston |

Military Service:

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|---|---|
| 1973-75 | Lieutenant Commander, Medical Corps, U.S. Navy, Chief of Pediatrics, Naval Hospital, Portsmouth, New Hampshire |

Faculty Appointments:

| | |
|---|---|
| 1978-80 | Instructor in Pediatrics, Department of Pediatrics, Harvard Medical School |
| 1978-80 | Assistant in Cardiology, Department of Cardiology The Children's Hospital, Boston |
| 1980-86 | Associate in Cardiology, Department of Cardiology The Children's Hospital, Boston |
| 1980-86 | Assistant Professor of Pediatrics, Department of Pediatrics, Harvard Medical School |
| 1981-86 | Associate in Cardiology, Laboratory of Human Biochemistry, The Children's Hospital, Boston |
| 1986-97 | Associate in Cardiology, Department of Pediatrics, University of Michigan Medical School, C.S. Mott Children's Hospital |
| 1986-90 | Associate Professor of Pediatrics, Department of Pediatrics, University of Michigan Medical School |
| 1986-91 | Associate Professor of Pharmaceutics, Department of Pharmaceutics University of Michigan School of Pharmacy |

| | |
|---|---|
| 1990-97 | Professor of Pediatrics, Department of Pediatrics University of Michigan Medical School |
| 1991-97 | Professor of Pharmaceutics, Department of Pharmaceutics, University of Michigan Medical School |
| 1997 | Senior Member, Joseph Stokes Research Institute, The Children's Hospital of Philadelphia |
| 1997 | Member, Institute for Medicine and Engineering, University of Pennsylvania |
| 1997 | Member, Institute for Human Gene Therapy, University of Pennsylvania |
| 1997 | Professor of Pediatrics, Tenure Track, The University of Pennsylvania School of Medicine |
| 1998 | The William J. Rashkind Chair in Pediatric Cardiology, The Children's Hospital of Philadelphia |
| 1999 | Professor of Pharmacology, The University of Pennsylvania School of Medicine |

Hospital and Administrative Appointments;

Harvard Medical School:

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|---|---|
| 1983-86 | Preventive Cardiology Clinic (Director), The Children's Hospital, Boston |

University of Michigan

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| 1986 | Director, Pediatric Cardiology Biochemistry Laboratories |
| 1987-92 | Research Advisory Committee, Department of Pediatrics |
| 1988-91 | Faculty Senate |
| 1988-89 | Chairman, Search Committee for the Directorship of Pediatric Neurology |
| 1991 | Office of the Vice President for Research's (OVPR) Advisory Committee on Improving the Quality and Cost Effectiveness of OVPR's Operations |

Other

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|---|---|
| 1995 | Tenure Review Committee, The Hebrew University of Jerusalem |

The Children's Hospital of Philadelphia

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|---|---|
| 1997 | Senior Physician |
| 1997 | Senior Member, Joseph Stokes Jr. Research Institute |
| 2001 | Director, Cardiology Research Laboratories, Children's Hospital of Philadelphia |

Specialty Certification:

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|---|---|
| 1975 | American Board of Pediatrics |

Licensure:

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|---|---|
| 1986 | Michigan |
| 1997 | Pennsylvania |

Awards, Honors and Membership in Honorary Societies:

| | |
|---|---|
| 1965 | Phi Eta Sigma |
| 1966 | Phi Beta Kappa |
| 1982 | American Academy of Pediatrics Young Investigator Award |
| 1983 | Society for Pediatric Research |
| 1985 | Whitaker Health Sciences Foundation Award |
| 1986 | Established Investigator of the American Heart Association |
| 1987 | Rackham International Fellowship with The Hebrew University of Jerusalem |
| 1987-90 | Investigator, United States — Israel Binational Science Foundation |
| 1988 | Ebert Prize of the American Pharmaceutical Association |
| 1988 | American Pediatric Society |
| 1990 | Alpha Phi Foundation Cardiovascular Research Prize |
| 1992 | Clemson Award, Society for Biomaterials |
| 1994 | Fellow, National Academy of Biomaterials Science and Engineering |
| 1995 | Forchheimer Sabbatical Professor, The Hebrew University of Jerusalem |
| 1995 | University of Michigan Technology Award |
| 1996 | University of Michigan Technology Award |
| 1996 | Honorary Professorship, Institute of Biomedical Engineering of Peking Union Medical College and Chinese Academy of Medical Sciences |
| 1996 | Honorary Professorship, Cardiovascular Institute and Fu Wai Hospital, Chinese Academy of Medical Sciences |
| 1998 | Fellow, American Institute for Medical and Biological Engineering |
| 2000 | Member, John Morgan Society, University of Pennsylvania School of Medicine |
| 2001 | Discover Magazine Technology Award |
| 2002 | Children's Hospital of Philadelphia Technology Award |
| 2002 | Luigi Mastroianni Clinical Innovator Award, University of Pennsylvania School of Medicine |
| 2002 | Johnson and Johnson Focused Giving Program Award |

Memberships in Professional and Scientific Societies:

*National Societies:*

American Society for Artificial Internal Organs, Program Committee (1994)
World Congress Program Committee, International Society for Heart Research (1987-1989)
Second Jerusalem Conference on Pharmaceutical Sciences, Planning Committee (1995)
Controlled Release Society, International Program Committee, Nice, France (1994)
Third Jerusalem Conference on Pharmaceutical Sciences, Symposium Co-Chair (1995-96)
Executive Committee, International Society for Applied Cardiovascular Biology (1996-98)
Executive Board, American Society for Artificial Organs (1996-98)
4th Jerusalem Conference on Pharmaceutical Sciences, International Program Committee (1999)
Member at Large, Executive Board, Society for Biomaterials (2001-02)

*Local Societies*:

None

Research Grant Review Activity (Selected)

| | |
|---|---|
| 1993 | Site Visit Reviewer, Medical Research Council of Canada |
| 1993 | NHLBI Study Section on Cardiovascular Disease in Women |
| 1994 | Ad Hoc Reviewer, NIH Special Study Section on Biomaterials |
| 1998 | Member, NIH Special Study Section on Tissue Engineering and Biomimetics |
| 2001 | National Institutes of Health, Training Grant Study Section |
| 2001 | National Science Foundation, Special Study Section on Retroviral Gene Therapy Vectors |
| 2001 | National Institutes of Health, Special Study Section on Centers of Biomedical Research Excellence |
| 2001 | National Institutes of Health, Chair, Special Study Section on Cardiovascular Calcification |
| 2001 | Ad Hoc Member, NIH Pathology A Study Section. |
| 2001 | Member NHLBI Training Grant Study Section. |
| 2002 | Member, NIH Special Study Section on Tissue Engineering. |
| 2003 | Member, NHLBI Program Project Special Emphasis Panel |
| 2003 | Member, NIH Special Study Section on Tissue Engineering |
| 2003 | Member, Special Review Panel, Irish National Science Foundation |
| 2003 | External reviewer, Medical Research Council of Canada |

Business Development Activities:

| | |
|---|---|
| 1995 | Co-Founder, *Selective Genetics, Inc.* San Diego, CA |

Editorial Board Positions:

| | |
|---|---|
| 1992 | Editorial Board, Biomaterial-Living System Interactions (BIOMIR), Moscow |
| 1996-98 | Editorial Board, ASAIO Journal |
| 1996 | Guest Editor, Advanced Drug Delivery Reviews |
| 1998 | Editorial Board, Biomaterials |
| 1999 | Editorial Board, Pharmaceutical Research |

Editorial Board Reviewing Activity

American Journal of Pathology
Annals of Thoracic Surgery
ASAIO Journal
Biomaterials
Cancer Research
Cardiovascular Pathology
Circulation
Circulation Research
FASEB Journal
Gene Therapy
Human Gene Therapy

Journal of Biomaterial Science: Polymer Edition
Journal of Cardiovascular and Thoracic Surgery
Journal of Cardiovascular Pathology
Journal of Clinical Investigation
Journal of Heart Valve Disease
Journal of Microencapsulation
Journal of Pharmaceutical Science
Molecular Therapy
Nature Biotechnology
Pharmaceutical Research
Proceedings of the National Academy of Sciences
The Journal of Biomedical Materials Research
The Journal of Controlled Release

Academic Committees at the University of Pennsylvania and The Children's Hospital of Philadelphia:

| | |
|---|---|
| 1998 | Member, Committee on Appointments and Promotions, The Children's Hospital of Philadelphia |
| 1998 | Member, Stokes Lectureship Committee, The Children's Hospital of Philadelphia |
| 1998 | Member, I.R. B., The Children's Hospital of Philadelphia |
| 1999 | Program Director, NHLBI Institutional Research Service Award, Molecular Therapeutics for Pediatric Cardiology |
| 1999 | Member, Committee on Fetal Therapy, The Children's Hospital of Philadelphia |
| 1999 | Member, Medical Advisory Committee for the Foerderer Fund for Excellence, The Children's Hospital of Philadelphia |
| 1999 | Medical Advisory Subcommittee for the Foerderer Fund |
| 2000 | Member, Biomedical Coordination Committee (BEN@ PENN) University of Pennsylvania |
| 2000 | Chair, Committee on Appointments and Promotions, The Joseph Stokes Jr. Research Institute, The Children's Hospital of Philadelphia |
| 2002 | Oversight Committee, NIH Clinical Trial for Twin-Twin Transfusion |
| 2002 | Oversight Committee, NIH Clinical Trial for Fetal Meningomyclocele Repair |

Academic Committees at the University of Michigan:

| | |
|---|---|
| 1986-97 | Pediatric Cardiology Biochemistry Laboratories (Director) |
| 1986-97 | Attending Physician, Pediatric Cardiology |
| 1987-92 | Research Advisory Committee, Department of Pediatrics |
| 1988-91 | Faculty Senate |
| 1988-89 | Chairman, Search Committee for the Directorship of Pediatric Neurology |
| 1988-91 | University Committee on the Use and Care of Animals (UCUCA) |
| 1989-92 | Biomedical Research Council |
| 1989-92 | Director, Preventive Cardiology Clinic, C.S. Mott Children's Hospital |
| 1990-92 | Medical Student Fellowship Committee |

| | |
|---|---|
| 1990-97 | Pediatric Preventive Cardiology Clinic (Director) |
| 1990-93 | Board for Student Publications |
| 1991 | Office of the Vice President for Research's (OVPR) Advisory Committee on Improving the Quality and Cost Effectiveness of OVPR's Operations |
| 1991 | Department of Pediatrics First Annual Research Symposium |
| 1991 | Evaluation and Management of Valvular Insufficiency: New Approaches for the 90's, Department of Internal Medicine |
| 1991 | The Restenosis Summit III, Department of Internal Medicine |
| 1991 | Cardiovascular Research Center |
| 1991 | Newborn Care Internal Review Committee, Department of Pediatrics |
| 1992 | Search Committee for Chair of Biomaterials, Dental School |
| 1992-93 | Search Committee, Chief of Newborn Services, Department of Pediatrics |
| 1992-93 | Fund Raising Committee, Amnon Rosenthal Professorship, School of Medicine |
| 1994 | SCOR (NIH) in Rheumatoid Arthritis Internal Advisory Board |
| 1995 | Child Health Research Center Advisory Committee |
| 1995 | Materials Science Center Internal Advisory Committee, Office of the Vice President for Research |
| 1995 | Tissue Engineering Working Group, School of Medicine |
| 1996 | Michigan Congenital Heart Center Coordinating Council |
| 1996 | Research Advisory Council |
| 1996 | Search Committee, Pediatric Pulmonology |
| 1996 | Medical School Committee on Student Biomedical Research |

Major Teaching and Clinical Responsibilities at the University of Michigan

1986-1997 Attending Physician, Pediatric Cardiology, University of Michigan Hospitals

Lectures by Invitation (Since 1994):

| | |
|---|---|
| 1994 | "Polyurethane Calcification" — American Chemical Society Polymer Symposium, Ann Arbor, Michigan |
| 1994 | "Controlled Release for Arrhythmias" — Third European Symposium on Controlled Drug Delivery, The Netherlands |
| 1994 | "Clinical Controlled Release Systems" — Twenty-First International Symposium on Controlled Release of Bioactive Materials, Nice, France |
| 1994 | "Cardiac Valve Bioprosthesis" — ASAIO/NIH Cardiovascular Science and Technology Conference, Washington, D.C. |
| 1995 | Invited Participant, National Heart, Lung and Blood Institute Workshop on Tissue Engineering, held at 1995 Gordon Conference on Biomaterials, Holderness, New Hampshire |
| 1996 | "Mechanistic Approaches for Preventing Bioprosthetic Calcification" — International Society for Applied Cardiovascular Biology Fifth Biennial Meeting, Manchester, England |
| 1996 | "Polymers in Medicine" — Academy of Medical Sciences, People's Republic of China |
| 1996 | "Advanced Therapies for Cardiac Valve Disease" — National Heart, Lung and Blood Institute Workshop on Heart Valve Prostheses, Washington, D.C. |

| | |
|---|---|
| 1996 | "How to Prevent or Mitigate Dystrophic Calcification" — XVIII Congress of the European Soci ty of Cardiology, Birmingham, England |
| 1996 | "Cardiac Controlled Release Implants for Arrhythmias" — Third Jerusalem Conference on Pharmaceutical Sciences and Clinical Pharmacology, Jerusalem, Israel |
| 1996 | "Cardiac Drug Delivery Mechanisms" — Cardiology Grand Rounds, The University of Michigan, Ann Arbor, Michigan |
| 1997 | "Current Progress in Anticalcification for Bioprosthetic and Polymeric Heart Valves" — The University of Michigan Medical School, Ann Arbor, Michigan |
| 1997 | "Differential Calcification of Cusps and Aortic Wall of Failed Stented Porcine Bioprosthetic Valves" — The University of Michigan Medical Center, Ann Arbor Michigan |
| 1997 | "Synergistic Inhibition of Calcification of Porcine Aortic Root with Preincubation in FeC13 and Alpha-Amino Oleic Acid in a Rat Subdermal Model, Medtronic Heart Valve, Irvine, CA. |
| 1997 | "Arterial Nanoparticle Administration for Restenosis", Presented at the 24th International Symposium on Controlled Release of Bioactive Materials Stockholm, Sweden |
| 1997 | "Valvular Drug Delivery", Presented at the Conference on Formulations and Drug Delivery, sponsored by the American Chemical Society and the Controlled Release Society, La Jolla, California |
| 1997 | "Sustained Release Nanoparticles for Restenosis", Presented at the American Association of Pharmaceutical Science, Boston |
| 1997 | "Delivery Enhancers', 4th European Conference on Cardiovascular Drug Delivery, Geneva, Switzerland. |
| 1997 | "Technological Advances for Prosthetic Heart Valves", Shaping the Future of Cardiac Surgery, Paris, France. |
| 1998 | "Anticalcification Treatment: State of the Art" Endocarditis and Thrombogenecity in Patients with Prosthetic Valves, Helsinki, Finland |
| 1999 | Bioprosthetic Heart Valve Calcification: Mechanisms and Prevention, Epic Heart Valve Clinical Launch, Ivalo, Finland |
| 1999 | Clinical use of the Ethanol Pretreated Bioprosthesis, BioCor Institute Belo Horizonte, Brazil. |
| 2000 | "Mechanisms of Cardiovascular Calcification". Pharmacology Seminar, University of Pennsylvania School of Medicine |
| 2000 | "Controlled Release Stents". Lifeline Foundation, Washington, D.C. |
| 2000 | "Gene Delivery Systems" XII International Symposium on Atherosclerosis. Stockholm, Sweden. |
| 2001 | Calcification Resistance with Aluminum-Ethanol Treated Porcine Aortic Valve Bioprostheses. Stentless Heart Valve Meeting, San Diego. |
| 2001 | Inhibition of Cusp and Aortic Wall Calcification in Ethanol and Aluminum Treated Heart Valves in Sheep. Society for Heart Valve Disease, London, United Kingdom. |
| 2001 | Delivery Stents. Boston Scientific. Natick, Massachusetts |
| 2001 | Bench Research in the 1970's. The Alexander Nadas Memorial Symposium. |

Children's Hospital, Boston

2002 Heart Valve Disease, UWEB Symposium, University of Washington, Seattle

2002 Gene Delivery Systems, Genzyme Corporation, Boston, Massachusetts

2002 Antibody-mediated Gene Delivery, Cystic Fibrosis Research Foundation, Philadelphia, PA

2002 Reverse Gene Therapy, Johnson & Johnson, New Brunswick, NJ

2003 Site Specific Gene Therapy, National Institutes of Standards and Technology, Gaithersburg, MD

2003 TGA Preclinical Strategies: St. Jude Medical, St. Paul, MN

Bibliography:

Research Publications, peer reviewed:

1. Levy, R.J., Rosenquist, G.C.: Anatomical variations in tricuspid atresia: report of two cases with previously undescribed lesions, Johns Hopkins Medical Journal 126:177-183, 1970.
2. Krovetz, L.J., Simon, A.L., Levy, R.J., Tift, W.: Effects of angiographic contrast media on left ventricular function, Johns Hopkins Medical Journal 127:172-179, 1970.
3. Rosenquist, G.C., Levy, R.J., Rowe, R.D.: Right atrial-left ventricular relationships in tricuspid atresia, American Heart Journal 80:493-500, 1970.
4. Levy, R.J., Rosenthal, A., Freed, M.D., Smith, C.D., Eraklis, A., Nadas, A.S.: Persistent pulmonary hypertension in an infant with congenital diaphragmatic hernia successfully managed with Tolazoline, Pediatrics 60:740-742, 1977.
5. Levy, R.J., Rosenthal, A., Fyler, D.C., Nadas, A.S.: Birthweight of infants with congenital heart disease, American Journal of Diseases of Children 132:249-257, 1978.
6. Levy, R.J., Rosenthal, A., Castaneda, A.R., Nadas, A.S.: Growth after surgical repair of d-transposition of the great vessels with intact ventricular septum, Annals of Thoracic Surgery 25:225-232, 1978.
7. Levy, R.J., Rosenthal, A., Miettinen, O.: Determinants of growth in patients with ventricular septal defect, Circulation 57:793-799, 1978.
8. Levy, R.J., Lian, J.B.: Gammacarboxyglutamate excretion and warfarin therapy, Clinical Pharmacology and Therapeutics 25:562-571, 1979.
9. Levy, R.J., Lian, J.B., Gallop, P.M.: Atherocalcin, a gammacarboxyglutamic acid containing protein from atherosclerotic plaque, Biochemical and Biophysical Research Communications 91, 41-49, 1979.
10. Levy, R.J., Zenker, J.A., Lian, J.B.: Vitamin K-dependent calcium binding proteins in aortic valve calcification, Journal of Clinical Investigation 65:563-566, 1980.
11. Sanders, S.P., Levy, R.J., Freed, M.D., Norwood, W.I., Castaneda, A.R.: Use of Hancock porcine xenografts in children and adolescents, American Journal of Cardiology 46:429-438, 1980.
12. Lian, J.B., Levy, R.J., Bernhard, W.F., Szycher, M.: LVAD mineralization and gammacarboxyglutamic acid containing proteins in normal and pathologically mineralized tissues, Transactions of the American Society of Artificial Internal Organs 27:683-689, 1981.
13. Fishbein, M., Levy, R.J., Ferrans, V.J., Dearden, L.C., Nashef, A., Goodman, A.P., Carpentier, A.: Calcification of cardiac valve bioprostheses. Biochemical, histologic, and ultrastructural observations in a subcutaneous implantation model system, Journal of Thoracic and Cardiovascular Surgery 83:602-609, 1982.
14. Levy, R.J., Gundberg, C.M., Scheinman, R.: The identification of the vitamin K-dependent bone protein osteocalcin as one of the carboxyglutamic acid containing proteins present in calcified atherosclerotic plaque and mineralized heart valves, Atherosclerosis 46:49-56, 1983.
15. Levy, R.J., Zenker, J.A., Bernhard, W.F.: Porcine bioprosthetic valve calcification in bovine left ventricle to aorta shunts: studies of the deposition of vitamin K-dependent proteins, Annals of Thoracic Surgery 36:187-192, 1983.

16. Levy, R.J., Schoen, F.J., Levy, J.T., Nelson, A.C., Howard, S.L., Oshry, L.J.: Biologic determinants of dystrophic calcification and osteocalcin deposition in glutaraldehyde-preserved porcine aortic valve leaflets implanted subcutaneously in rats, American Journal of Pathology 113:143-155, 1983.
17. Levy, R.J., Schoen, F.J., Howard, S.L.: Mechanism of calcification of porcine bioprosthetic aortic valve cusps: role of T-lymphocytes, American Journal of Cardiology 52:629-631, 1983.
18. Sherman, F.S., Schoen, F.J., Hawley, M., Nichols, J., Levy, R.J.: Collagen cross-links: a critical determinant of bioprosthetic heart valve calcification, Transactions of the American Society of Artificial Internal Organs XXX:577-581, 1984.
19. Levy, R.J., Hawley, M.A., Schoen, F.J., Lund, S.A., Liu, P.Y.: Inhibition by diphosphonate compounds of calcification of porcine bioprosthetic heart valve cusps implanted subcutaneously in rats, Circulation 71:349-356, 1985.
20. Schoen, F.J., Levy, R.J., Nelson, A.C., Bernhard, W.F., Nashef, A., Hawley, M.: Onset and progression of experimental bioprosthetic heart valve calcification, Laboratory Investigation 52:523-532, 1985.
21. Levy, R.J., Wolfrum, J., Schoen, F.J., Hawley, M.A., Lund, S.A., Langer, R.: Inhibition of calcification of bioprosthetic heart valves by local controlled-release diphosphonate, Science 228:190-192, 1985.
22. Nelson, A.C., Schoen, F.J., Levy, R.J.: SEM methodology for study of the pathophysiology of calcification in bioprosthetic heart valves, Scanning Electron Microscopy I:209-213, 1985.
23. Levy, R.J., Golomb, G., Wolfrum, J., Lund, S.A., Schoen, F.J., Langer, R.: Local controlled-release of diphosphonates from ethylenevinylacetate matrices prevents bioprosthetic heart valve calcification,
Transactions of the American Society of Artificial Internal Organs 31:459-463, 1985.
24. Levy, R.J., Schoen, F.J., Sherman, F.S., Nichols, J., Hawley, M.A., Lund, S.A.: Calcification of subcutaneously implanted type I collagen sponges: effects of formaldehyde and glutaraldehyde, American Journal of Pathology 122:71-82, 1986.
25. Golomb, G., Dixon, M., Smith, M.S., Schoen, F.J., Levy, R.J.: Inhibition of bioprosthetic heart valve calcification by sustained local delivery of Ca and Na diphosphonate via controlled release matrices, Transactions of the American Society of Artificial Internal Organs 32:587-590, 1986.
26. Levy, R.J., Howard, S.L., Oshry, L.J.: Carboxyglutamic acid (Gla) containing proteins of human calcified atherosclerotic plaque solubilized by EDTA: molecular weight distribution and relationship to osteocalcin, Atherosclerosis 59:155-160, 1986.
27. Schoen, F.J., Tsao, J., Levy, R.J.: Calcification of bovine pericardium used in cardiac valve bioprostheses: role of glutaraldehyde-modified structural components in bioprosthetic tissue mineralization, American Journal of Pathology 123:134-145, 1986.
28. Jonas, R.A., Schoen, F.J., Levy, R.J., Castaneda, A.R.: Biological sealants and knitted Dacron-porosity and histological comparisons of vascular graft materials with and without collagen and fibrin glue pretreatments, Annals of Thoracic Surgery 41:657-663, 1986.
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# Background & Hypothesis:

- A missense ion channel mutation of Mink-related protein-1 (MiRP-1 Q9E) is associated with a clarithromycin dependent form of the Long QT syndrome (Abbott et al. *Cell* 99:175-87, 1999)
- Q9E results in prolonged inward K-rectifier currents (Ikr) compared to the wild type channel (WT), but only in the presence of the antibiotic clarithromycin. (Abbott et al. *Cell* 99:175-87, 1999)
- It is hypothesized that site specific overexpression in the atrium of Q9E with subsequent clarithromycin administration could result in controllable anti-arrhythmia gene therapy

# The rationale for the use of Q9E Gene Vectors:

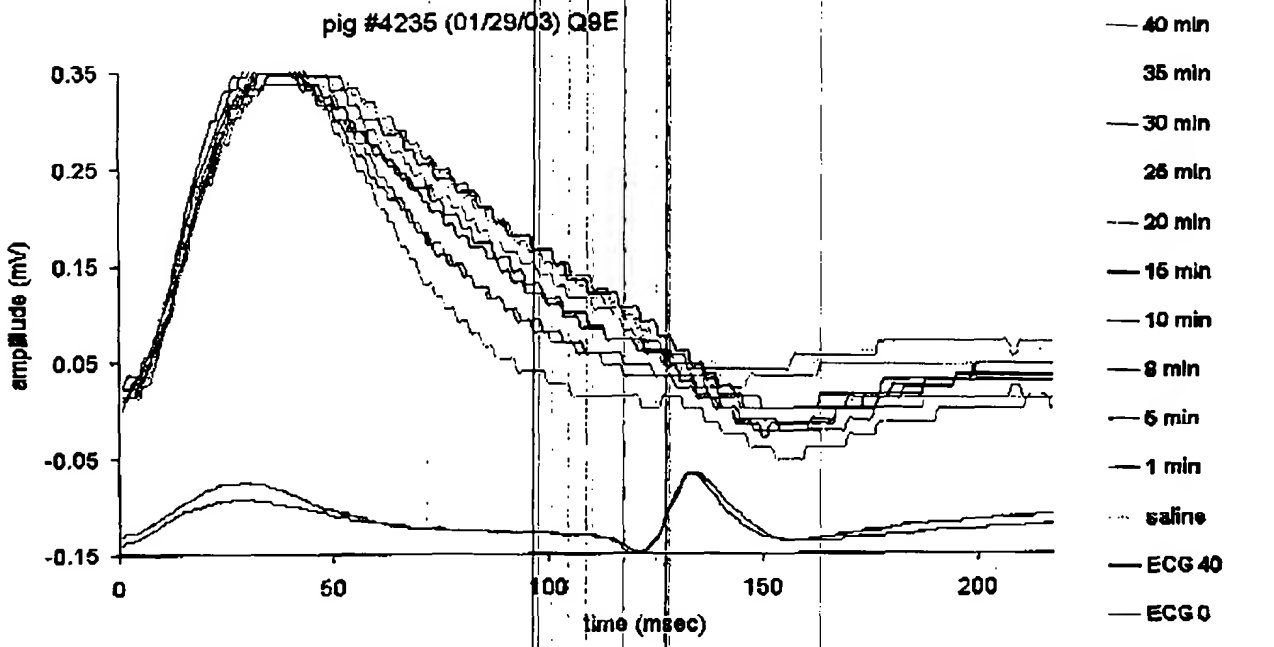
- Electrophysiologic effects comparable to class III anti-arrhythmic agents, that are commonly used to treat atrial and ventricular arrhythmias
- EP effects modulated by clarithromycin administration
- Atrial expression would be localized, and would not be expected to be associated with ventricular pro-arrhythmic effects

The Potassium Channel.
4 pore-forming proteins
(α subunits)
"accessory"
β subunit
exterior
interior
Wild-type
Channel,&
its mutant,
Q9E, used in
this study
Examples:
α- subunit
KCNQ1
HERG
Kv3.4
MiRP
MinK
MiRP1
MiRP2
Abbott & Goldstein, *Mol. Intervent.* (June 2001)

# Methods:

1. Creation and characterization of bi-cistronic plasmid DNA vectors for MiRP-1 & Q9E
2. Establish stable cell lines (HEK293) overexpressing the vectors (using antibiotic selection w gentamycin)
3. Single cell electrophysiology (patch clamp) studies
4. Large animal (pig) studies: plasmid DNA injection to the right atrium with endpoints of expression and electrophysiologic changes (monophasic action potential, MAP).

# #4235 Q9E

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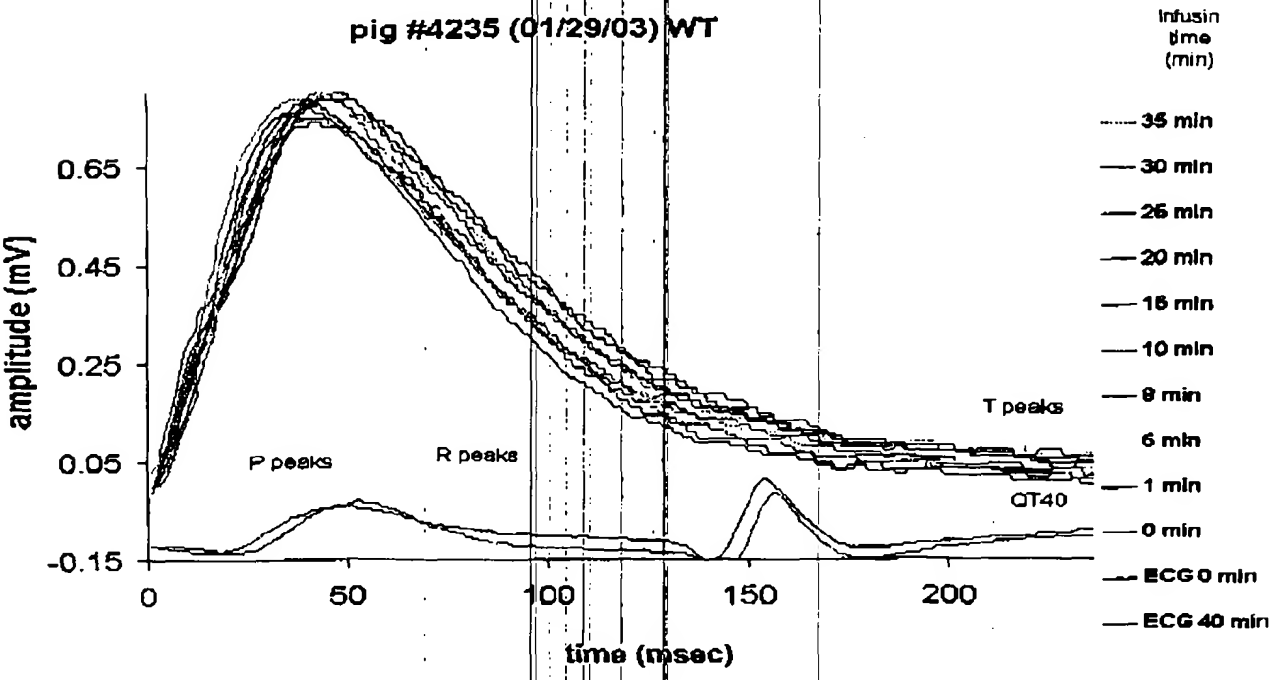

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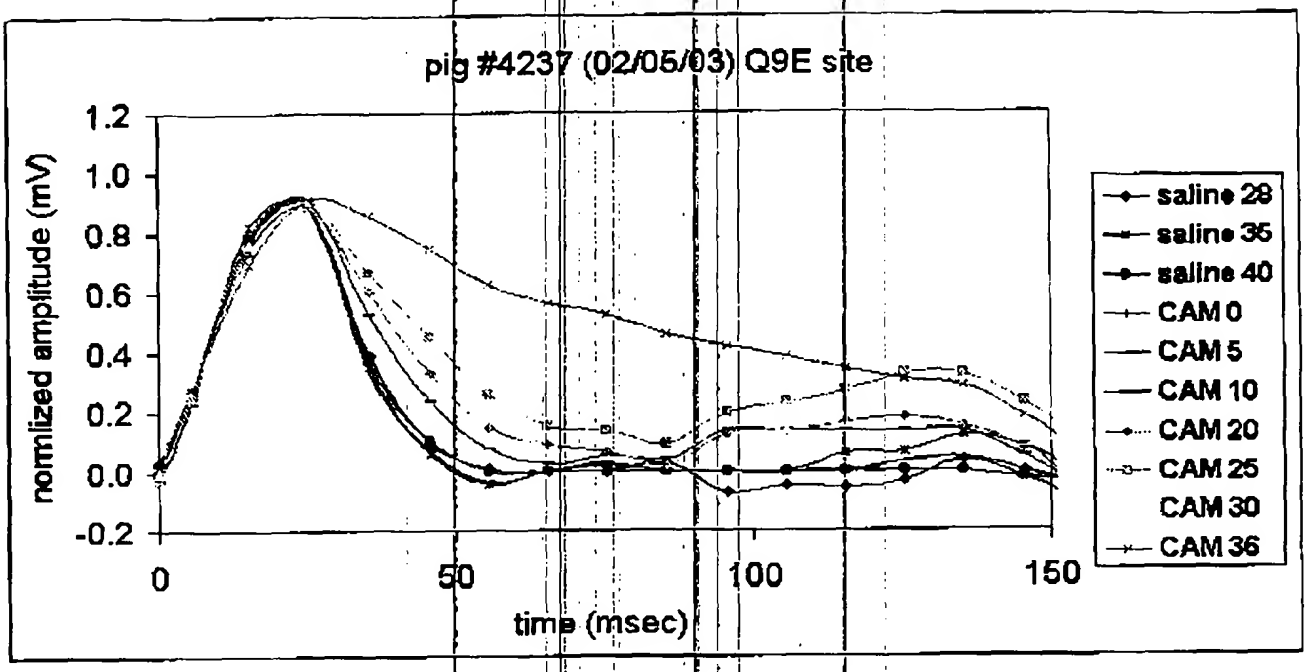


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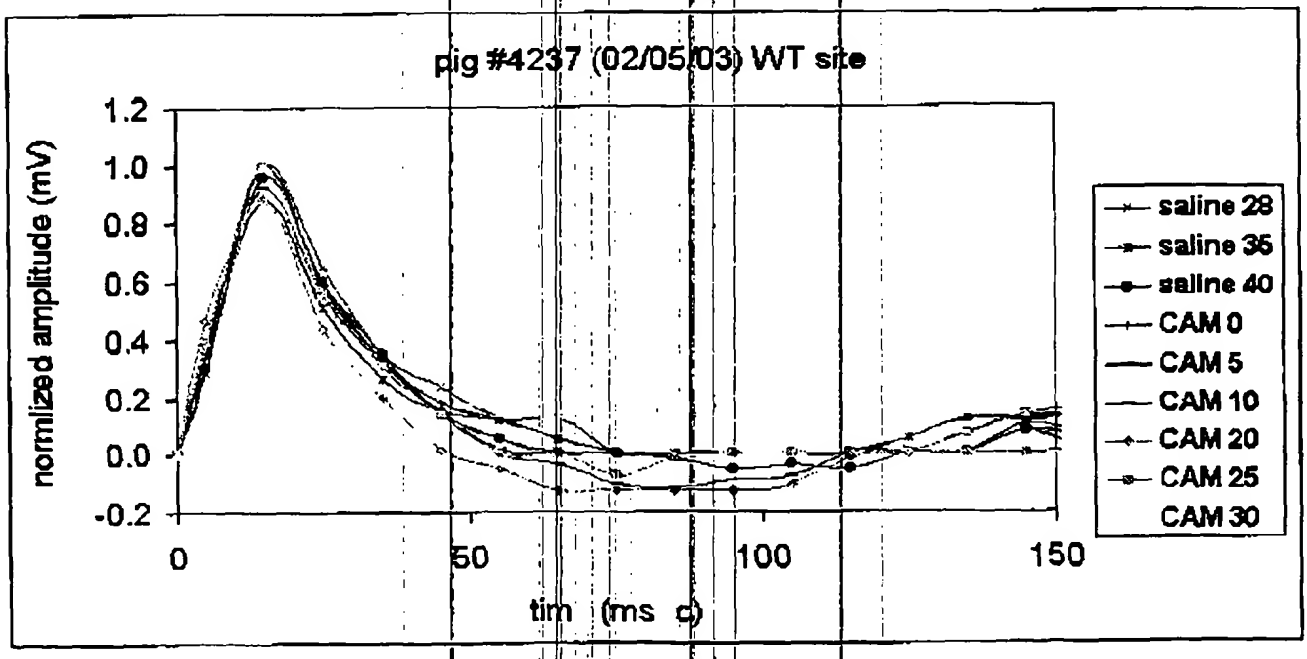

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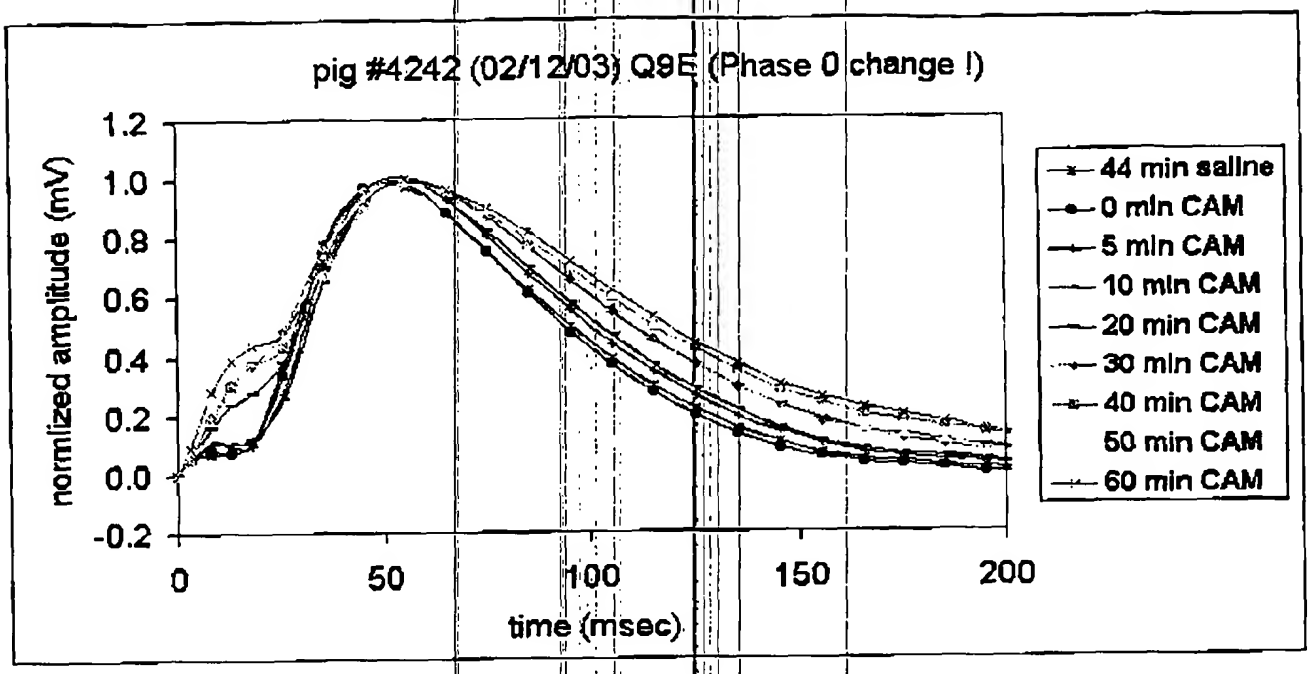


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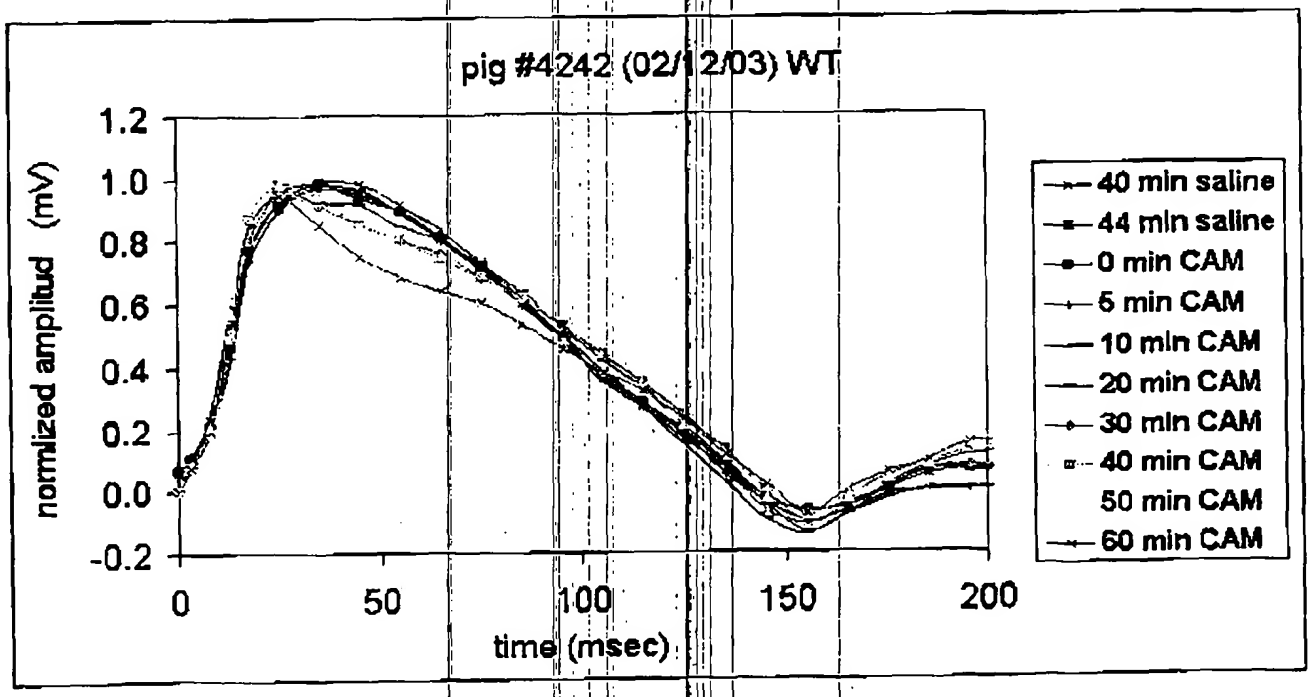

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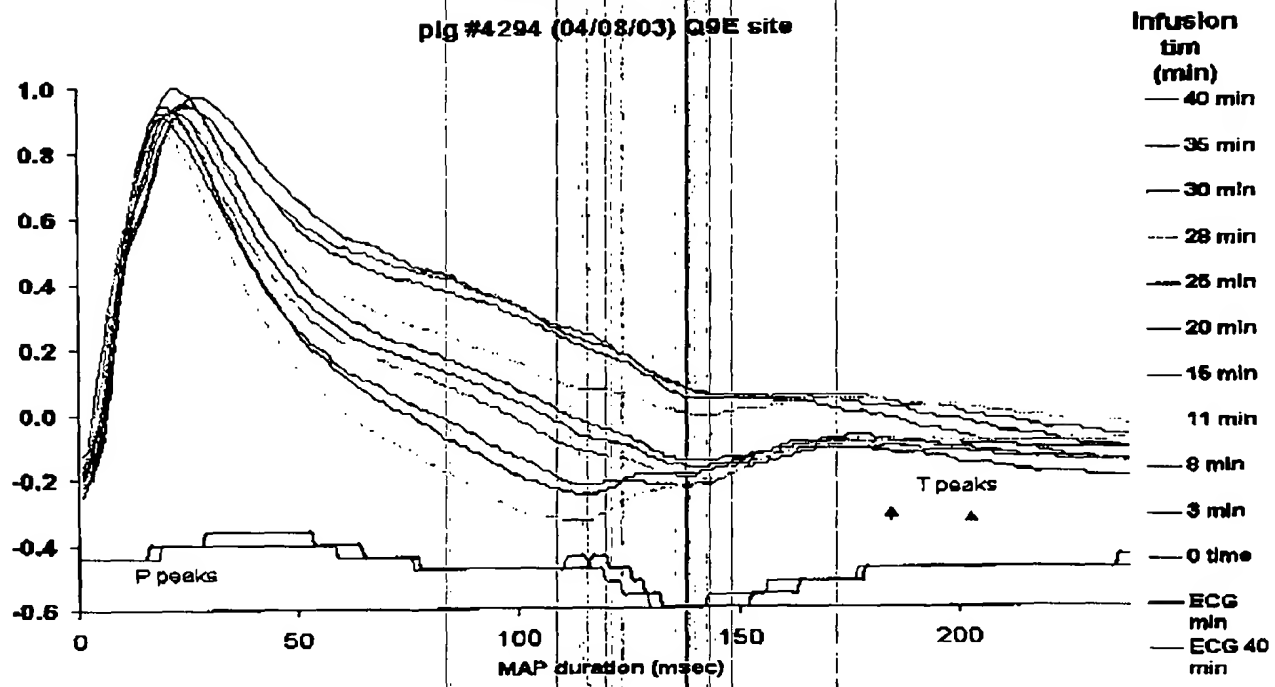


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